Project title:	Evaluating potential new fungicides for the control of *Narcissus* basal rot in bulb and plant tests
Project number:	BOF 74a
Project leader:	**John Clarkson** Warwick Crop Centre School of Life Sciences University of Warwick Wellesbourne Campus Wellesbourne, Warwick CV35 9EF
Report:	Final report, July 2014
Previous report:	None
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Location of project:	Warwick Crop Centre
Industry Representative:	Mark Clark Grampian Growers Ltd
Date project commenced:	01/06/2013
Date project completed (or expected completion date):	31/08/2014

The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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Signature: John Clarkson Date: 21/07/14

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GROWER SUMMARY

Headline

The fungicides Orius (tebuconazole), Mirage (prochloraz) and Storite (thiabendazole) gave good control of Fusarium basal rot when applied to *Narcissus* bulbs using standard hot water treatment. Tebuconazole was the most effective and represents an effective alternative active to the currently approved thiabendazole. HDC are currently pursuing an EAMU for tebuconazole, while EU approval for thiabendazole will be lost at the end of 2015. The fungicide HDC F165 was the most effective of three triazoles selected as potential new actives and tested for their effect on *Fusarium* on agar.

Background

The UK *Narcissus* industry continues to experience increasing levels of basal rot caused by *Fusarium oxysporum f.sp. narcissi (*FON*)*, in particular with many of the yellow varieties. Growers rely on only two fungicide active ingredients for the control of this disease; chlorothalonil and thiabendazole, both of which are available for dipping bulbs including by hot-water treatment (HWT) through EAMUs. This situation leaves the industry vulnerable to a loss of disease control options as a result of legislation or commercial considerations.

A previous HDC project (BOF 74) identified new fungicides which could suppress the growth of FON isolates on agar, the most effective of which were prochloraz (an imidazole) and tebuconazole (a triazole). Results for the currently approved fungicides chlorothalonil and thiabendazole showed that the former was relatively ineffective except at the higher concentrations, while the latter failed to adequately reduce growth of two of the eight FON isolates tested, suggesting pathogen tolerance to this active ingredient.

The main aim of this project was to evaluate the protectant efficacy of prochloraz and tebuconazole against FON and compare with the current industry standards thiabendazole and chlorothalonil using small-scale HWT of *Narcissus* bulbs which were subsequently planted in growing medium artificially inoculated with the pathogen. A biological control agent applied to HW treated bulbs was also tested. The potential for curative activity of the above fungicides was also evaluated by HWT of a *Narcissus* bulb stock naturally infected with FON in a preliminary experiment. Three triazole fungicides not previously tested on agar in BOF 74 were also tested for their ability to reduce growth of FON isolates on agar.

Summary

Fungicide testing on agar

Three triazole fungicides (HDC F163, F164 and F165) were selected following consultation with growers and manufacturers and tested against eight pathogenic FON isolates representing different morphology groups and locations identified in BOF 74. Agar plugs from cultures of each FON isolate were placed on agar plates amended with each fungicide at rates of 1, 5, 10, 20, 50 and 100 ppm a.i. L^{-1} (mg L^{-1}) and growth recorded. The minimum fungicide concentration that inhibited mycelial growth by 50 and 95% (MIC50, MIC95) was also calculated for each isolate/fungicide combination.

All three triazole fungicides reduced growth of all FON isolates on agar at all concentrations tested compared to the control cultures (no fungicide, Fig. 1). HDC F165 had greater activity than the other products at all concentrations. MIC50 values were <1ppm for all three fungicides while HDC F165 had the lowest MIC95 value of 3.6 ppm which was comparable with the most effective fungicides tested in BOF 74 (Mirage - prochloraz; Orius - tebuconazole, Agate, prochloraz + tebuconazole).

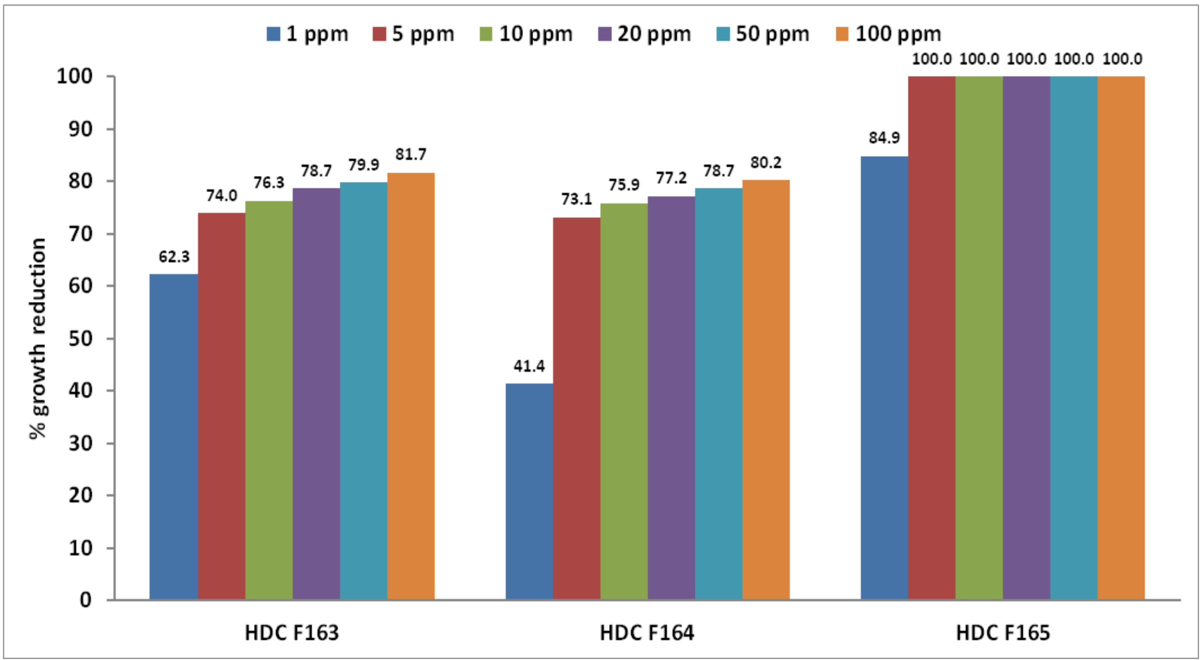

Figure 1. Percentage growth reduction of *F. oxysporum* f.sp. *narcissi* (FON) for six concentrations of three triazole fungicides on agar compared to untreated control (no fungicide). Data are means over the eight FON isolates tested.

Fungicide testing on *Narcissus* bulbs/plants

Two stocks of *Narcissus* bulbs with low (1%) and high incidence (9%) and severity of basal rot were identified for use in a 'protectant' and a preliminary 'curative' experiment respectively. For both experiments, four fungicides comprising the potential two new fungicides Mirage (prochloraz) and Orius (tebuconazole) and the two currently approved products Storite (thiabendazole) and Bravo (chlorothalonil) were used for HWT of Narcissus bulbs in September 2013. Mirage and Orius were both used at concentrations of 500 ppm a.i. while Storite and Bravo were used at currently recommended rates of 500 ppm a.i. and 275 ppm a.i. (ppm = mg L^{-1}) respectively, equivalent to 1.25 L and 1.0 L of product per 1000 L in HWT respectively.

In the curative experiment, following HWT with fungicides, the *Narcissus* bulbs (9% basal rot initially) were incubated for 60 days at 25°C after which basal rot was assessed on bisected bulbs using a 10-point severity scale. For the protectant experiment, fungicide-treated bulbs were planted in pots in a *Narcissus* growing medium inoculated with two levels of FON (low / high) and placed in a frost free glasshouse. HWT bulbs (no fungicide) were also treated with the biocontrol agent HDC F184. Plant growth was assessed February-April 2013 and bulbs assessed for basal rot in early June.

Incidence of basal rot in *Narcissus* bulbs at the end of the curative experiment was 19, 20, 57 and 60% for Bravo, Orius, Mirage and Storite respectively compared to 55% in the HWT no fungicide control. By comparison, incidence of basal rot in 40 spare bulbs (from the same lot) which had not undergone either HW or fungicide treatment was 0%. This latter result was unexpected as initial assessment of incidence was 9% but this may have been due to the relatively small number of bulbs used in the experiment. Mean basal rot severity scores were 0.6, 1.2, 2.0 and 4.1 for Bravo, Orius, Mirage and Storite respectively compared to 4.8 for the HWT no fungicide control and 0.0 for bulbs without HWT or fungicide. By comparison, the percentage of bulbs with low basal rot severity was 87-95% for Bravo, Orius and Mirage but was much lower for Storite at 55% (Fig. 2). The percentage of bulbs with low basal rot severity was 60% for the HWT control bulbs (no fungicide, Fig. 2). Hence, in contrast to Storite, the three fungicides Bravo, Orius and Mirage reduced basal rot development compared to the HWT control indicating that these products have some value in treating bulb stocks known to be contaminated with FON. The results also suggested that for bulbs not treated with fungicide, the HWT process appeared to promote FON infection. Although the experimental approach here attempted to test curative activity, it is unlikely that these fungicides can prevent disease development in bulbs with existing internal FON infection at the time of treatment as evidenced by the general increase in FON incidence from the original value of 9%. The observed activity is probably associated with a preventative mode of action where spores carried on, or just within, the basal plate are killed and any bulb to bulb disease spread is inhibited. Nevertheless these preliminary results suggest that fungicide treatment of bulb stocks with high initial FON incidence may be worthwhile and requires further investigation in a fully replicated experiment.

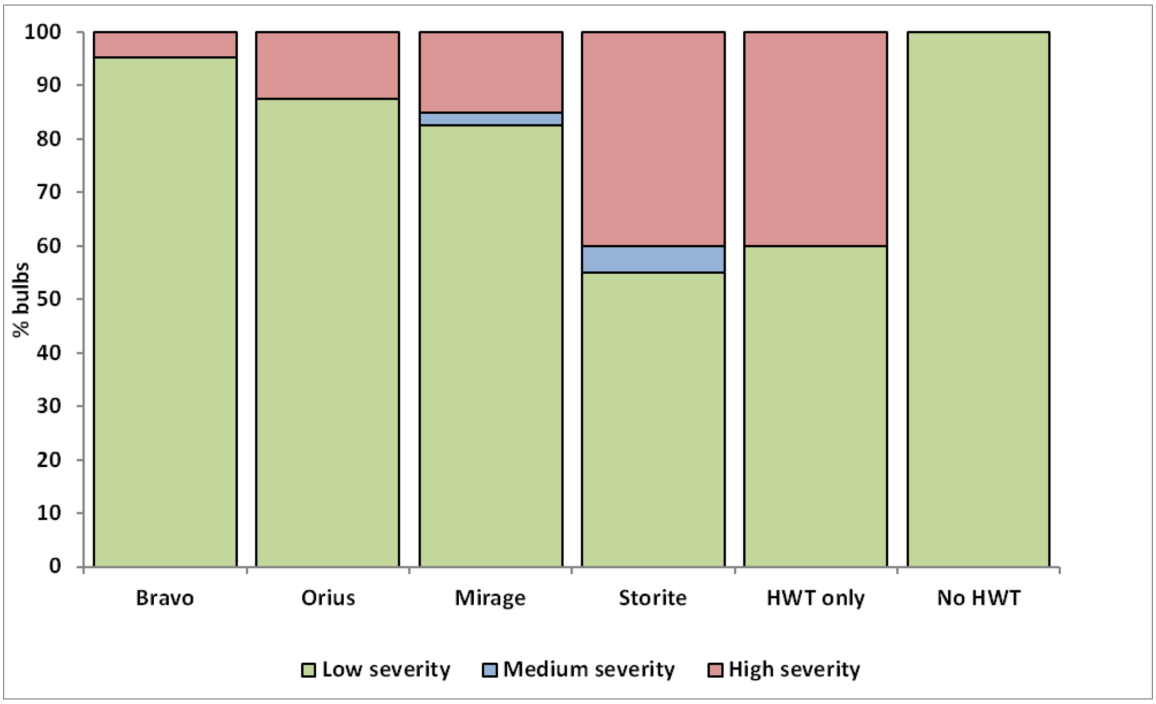

Figure 2. Percentage of *Narcissus* bulbs with low, medium and severe basal rot severity from a bulb stock with high initial disease incidence (9%) after HWT with different fungicides and incubation at 25°C for 60 days. HWT = hot water treatment only (no fungicide), No HWT = no hot water treatment (no fungicide).

In the protectant experiment, FON infection in the inoculated control treatments (bulbs not treated with fungicide and planted in growing medium with FON) was associated with reduced plant height and an increased number of yellowing/chlorotic *Narcissus* leaves. Some fungicides resulted in increased plant height while all of them significantly reduced the leaf chlorosis symptoms compared to the inoculated controls (low and high inoculum levels). However, the biocontrol treatment HDC F184 had no effect on these FON symptoms. Storite, Mirage and Orius significantly reduced basal rot severity for both FON inoculum levels ranging between 1.9 (Orius, low) and 3.9 (Storite, high) compared to 5.0 and 5.5 in the low and high inoculum untreated control treatments respectively (Fig. 3). Of these three fungicides, Orius was the most effective, resulting in the lowest disease severity score. However, Bravo did not significantly reduce basal rot severity while the biocontrol HDC F184 significantly increased disease severity at both FON levels compared to the inoculated controls. Storite, Mirage and Orius treatments also resulted in a significantly larger proportion of bulbs in the low basal rot severity category ranging from 45% (Storite, low/high) to 77.5% (Orius, low) compared to 22.5% in both the low and high inoculum untreated control treatments respectively (Fig. 4). Of the four fungicides, Orius was the most effective resulting in the highest proportion of bulbs in the low severity category (low, 77.5; high, 60.0; Fig. 3). Bravo only slightly increased the proportion of bulbs in the low disease severity category compared to the inoculated control treatments but this was not statistically significant and the biocontrol HD F184 was ineffective. Overall therefore, the protectant experiment showed that Orius, Mirage and Storite gave good control of basal rot with Orius (tebuconazole) being the most effective. Based on this, the HDC are now pursuing an EAMU for tebuconazole for use in HWT of Narcissus bulbs. This is timely as EU approval for thiabendazole will be lost at the end of 2015 and there are currently no plans from manufacturers to apply for re-registration of this product. Finally, although the biocontrol agent HDC F184 showed no activity against basal rot here, new biological products which are coming onto the market as well as other appropriate fungicide products should be evaluated in order to continue to develop a range of future options for basal rot control. A full review of the current status of chemical and biological options for basal rot control is given in the Science Section.

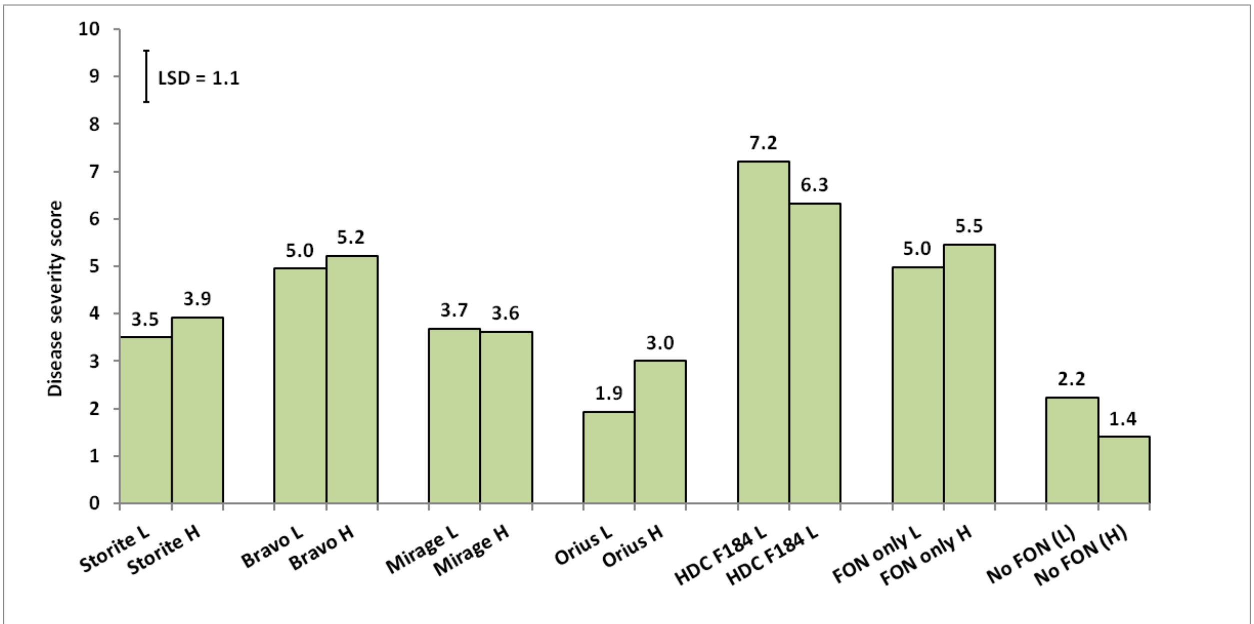

Figure 3. Effect of fungicides and the biocontrol agent HDC F184 on basal rot severity score for HWT *Narcissus* bulbs after planting in compost inoculated with *F. oxysporum* f.sp. *narcissi* (FON) at low (L) and high (H) inoculum levels. Data are means for 40 plants per treatment. Bar = Least Significant Difference (LSD, 5% level).

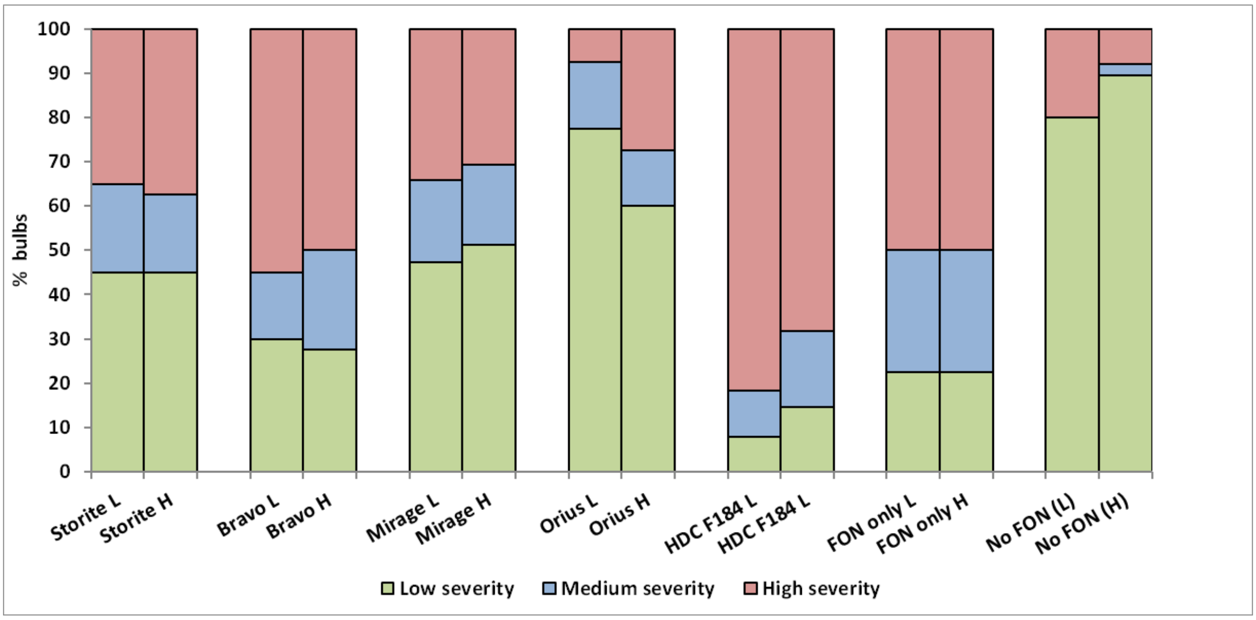

Figure 4. Effect of fungicides and the biocontrol agent HDC F184 on percentage of HWT *Narcissus* bulbs in different disease severity categories after planting in compost inoculated with *F. oxysporum* f.sp. *narcissi* (FON) at low (L) and high (H) inoculum levels. Data are means of for 40 plants per treatment.

Conclusions

- The fungicide HDC F165 was the most effective of three triazoles tested in reducing the growth of FON on agar and represents a potential alternative to the currently approved fungicides (Storite, Bravo) pending evaluation on bulbs in HWT.
- In a preliminary experiment, Bravo (chlorothalonil), Orius (tebuconazole) and Mirage (prochloraz) reduced basal rot development in a *Narcissus* bulb stock with initial high basal rot incidence (9%) but Storite (thiabendazole) had no effect. Results also suggested that HWT of bulbs promoted FON infection development.
- Orius (tebuconazole), Mirage (prochloraz) and Storite (thiabendazole) gave good control of basal rot for HWT bulbs planted in growing medium inoculated with FON with Orius (tebuconazole) being the most effective.
- HDC are currently pursuing an EAMU for tebuconazole for use in HWT of *Narcissus* bulbs.
- Approval for thiabendazole will be lost at the end of 2015 and there are currently no plans from manufacturers to apply for re-registration of this product except for possibly a co-formulation of thiabendazole and imazalil

- The biocontrol agent HDC F184 showed no activity against basal rot. However, new biological products which are coming onto the market should be evaluated in order to develop a range of options for basal rot control.

Financial Benefits

None at this time.

Action Points

- Continue to use approved thiabendazole and chlorothalonil based products for basal rot control alternated at full-rate in HWT every time a stock of bulbs is lifted and re-planted until new fungicides become available (Note that thiabendazole fungicides may not be applied more than once in any one year).
- Support the HDC in seeking EAMUs for products containing tebuconazole / prochloraz or both, identified as having good activity against Fusarium basal rot in this project.
- Support further work to identify and evaluate other fungicides with newer chemistry as well as biological and disinfectant approaches for basal rot control to insure against future legislation banning or restricting use of any of the above products.
- Support further work to examine alternative application methods if HWT with fungicides becomes unacceptable or restricts the range of products that can be used.
- Support further work to develop an integrated control programme for Fusarium basal rot incorporating chemical and biological treatments as well as development of resistant Narcissus varieties.

SCIENCE SECTION

Introduction

The UK *Narcissus* industry continues to experience increasing levels of basal rot caused by *Fusarium oxysporum f.sp. narcissi (*FON*)*, in particular with many of the yellow varieties (Mark Clark, personal communication, 2014). The disease is probably the most serious of this affecting *Narcissus* and growers rely on only two fungicide active ingredients for the control of this disease; chlorothalonil (e.g. as Bravo 500) and thiabendazole (as Storite Clear Liquid or Tezate 220 SL), both of which are available for dipping bulbs including by hot-water treatment (HWT) through EAMUs. This situation leaves the industry vulnerable to a loss of disease control options as a result of legislation or commercial considerations.

To address this, HDC project BOF 74 (Clarkson, 2012) was commissioned in 2011 to identify new fungicides which could suppress the growth of FON isolates on agar *in vitro*. Products based on prochloraz (an imidazole), tebuconazole (a triazole) or copper oxychloride were found to be most effective at suppressing FON growth. Results for the currently approved fungicides chlorothalonil and thiabendazole showed that the former was relatively ineffective except at the higher concentrations, while the latter failed to adequately reduce growth of two of the eight FON isolates tested, suggesting pathogen tolerance to this a.i.

The main aim of this project was to evaluate the protectant efficacy of prochloraz and tebuconazole against FON and compare it with the current industry standards thiabendazole and chlorothalonil using small-scale HWT of *Narcissus* bulbs which were subsequently planted in growing medium artificially inoculated with the pathogen. A biological control agent applied to hot-water (HW) treated bulbs was also tested. The potential for curative activity of the above fungicides was also evaluated by HWT of a *Narcissus* bulb stock naturally infected with FON in a preliminary experiment. Three triazole fungicides not previously tested on agar in BOF 74 were also tested for their ability to reduce growth of FON isolates on agar.

Materials and methods

Fungicide testing on agar

Three triazole fungicides (coded HDC F163, F164 and F165) were selected following consultation with growers and manufacturers and tested against eight pathogenic FON isolates representing different morphology groups and locations identified in BOF 74 (FON7, FON24, FON29, FON42, FON63, FON87, FON97 and FON139). Potato dextrose agar (PDA) plates were amended with each fungicide at rates of 1, 5, 10, 20, 50 and 100 ppm a.i. L^{-1} (mg L^{-1}) and a 5mm-diameter agar plug from an actively growing colony of each FON isolate placed in the centre. Fungal colony diameters (two perpendicular measurements per plate) were then measured after 7 days. The percentage growth reduction compared to the control plates (no added fungicide) was then calculated for each isolate/fungicide combination as 100-((diameter on fungicide amended medium/diameter on non-amended medium) ×100). Growth reduction was plotted against log fungicide rate and a regression line fitted. This enabled the minimum fungicide concentration that inhibited mycelial growth by 50 and 95% (MIC50, MIC95) to be calculated for each isolate/fungicide combination. Three replicate PDA plates were set up for each isolate/fungicide rate combination and an analysis of variance (ANOVA) was carried out on the angular-transformed growth reduction data using Genstat.

Fungicide testing on *Narcissus* bulbs/plants

Bulb stocks

Three stocks of *Narcissus* 'Carlton' with low (A), high (B) and very high (C) incidence of basal rot respectively were selected by Adrian Jansen, Lingarden Bulbs Ltd., from Cornish-grown stocks known to have received no post-lifting fungicide treatment. From each stock, approximately 54kg of bulbs (about 940 bulbs) of grade 12-14cm (circumference) were allocated for experiments. Following receipt of the bulbs from growers in August 2013, they were stored in bulb nets in a well-insulated, well ventilated warehouse until required.

One week before the start of experiments, the bulbs of each stock were well mixed, and 100-bulb samples were taken, excluding and discarding any obviously out-of-grade and mechanically damaged bulbs. To determine the incidence and severity of base rot in each stock, each bulb was bisected lengthways (taking care to divide the basal plate equally); where no basal rot was evident, the bulb halves were also bisected lengthways. The presence and severity of basal rot on each bulb was recorded on a 10-point scale (Table 1).

On the basis of these results the ‘A’ and ‘B’ stocks with 1 and 9% incidence of basal rot and with 99% and 94% of bulbs with low severity score (0-2) respectively were selected for use in ‘protectant’ and ‘curative’ experiments (see following sections). From the previously randomised bulb stocks, 560 bulbs were counted out from the ‘A’ stock and 200 from the ‘B’ stock, again excluding and discarding obviously out-of-grade and mechanically damaged bulbs. The bulbs were counted into lots of 40 which were placed in 1.2m-long length of tubular, knitted, nylon produce netting knotted at either end and labelled. The bulb lots remained in their nets for subsequent HWT until the bulbs were potted-up in FON-inoculated growing medium (‘protectant’ experiment) or examined after incubation at 25°C (‘curative’ experiment). They were transported to Warwick Crop Centre, Wellesbourne, on 23/09/13.

Narcissus bulb HWT and fungicide treatment

Four fungicides comprising the two potentially new fungicides Mirage (prochloraz) and Orius (tebuconazole), and the approved products Storite (thiabendazole) and Bravo (chlorothalonil) were used for HWT of *Narcissus* bulbs (stocks A and B). Final concentrations used in the HWT tanks were 500 ppm (mgL^{-1}) a.i. for Mirage and Orius while Storite and Bravo were added at the current recommended rates of 275 and 500 ppm a.i. respectively.

Five Clifton 38 L water baths were filled with 35 L of water with the addition of 3 ml of Agral (a non-ionic wetting agent) and allowed to reach the standard working temperature of 44.4°C (Fig. 5). Each fungicide under test was allocated a bath for all HWT of different bulb lots with the fifth bath exclusively used for all the control bulbs (no fungicide). When the baths had reached the working temperature, the appropriate amount of fungicide was added and mixed briefly. The bulb nets (40 bulbs each) were then lowered gently into the water baths, with a wire mesh placed on top to ensure the complete immersion of the bulbs during treatment. The baths were allowed to reach working temperature before commencing timing of the standard 3.25 hours HWT. At the end of the treatment, the bulb nets were immediately removed from the baths and the excess water drained. Nets were then placed into slatted trays and the bulbs dried by fans overnight at ambient temperature after which they were moved to a cool store (<20°C) before use. Lots of 40 *Narcissus* bulbs for the experiments were treated sequentially over four days (23-26/09/13) with baths being emptied, flushed out, refilled and wetter / fungicides added again for each run. Lots from the low basal rot stock A for the ‘protectant’ experiment were all treated before the high basal rot stock B used in the ‘curative’ experiment.

Figure 5. Water baths used for HWT of *Narcissus* bulbs with fungicides (left). Submerged bulbs in water bath during HWT (right).

Curative experiment

Narcissus bulbs (one net of 40 bulbs per treatment) from the basal rot infected stock B (9% incidence, low severity) which had undergone HWT with Mirage, Orius, Storite, Bravo or without fungicide were placed in individual trays, loosely covered with black plastic, and incubated for 60 days at 25°C in the dark in a controlled environment room. Basal rot was then assessed for each bulb by bisecting lengthways and the incidence and severity recorded on the 10-point scale (Table 1). No formal statistical analysis was carried out due to the provisional nature of the experiment and the relatively small number of infected bulbs that were available for treatment.

Table 1. Scoring scheme for assessing the severity of Fusarium basal rot in *Narcissus* bulbs.

Score	Severity	Zones affected by basal rot[1]
0	Low	None
1	Low	Spot or spots (up to 2mm-diameter) in base plate
2	Low	Small area of basal plate (up to 10%) but no spread to bulb scales
3	Medium	Up to 25% of basal plate area but no spread to bulb scales
4	Medium	Up to 50% of basal plate area but no spread to bulb scales
5	Medium	More than 50% of basal plate area but no spread to bulb scales
6	High	Start of spread from basal plate to bulb scales (up to 10% of scale area)
7	High	Up to 25% of bulb scale area
8	High	Up to 50% of bulb scale area
9	High	More than 50% of bulb scale area
10	High	Whole bulb (or virtually whole bulb) (includes dried, 'mummified' bulbs)

[1] Disease scores of 1-5 relate to basal rot in the base plate only; scores 6-10 relate to rot in bulb scales and are irrespective of percentage of basal plate affected.

Protectant experiment

F. oxysporum inoculum

FON isolate FON139 (previously identified in BOF 74 as being highly pathogenic) was grown on PDA for approx. four days at 20°C to produce actively growing cultures. Agar plugs (10 x 5 mm) from the leading edge of the colonies were then used to inoculate a sterile mix of 250 g *Narcissus* growing medium (see below), 33.3 g wheat bran and 117 ml water contained in 1 L flasks .which were incubated at 25°C in the dark for approx. three weeks. To quantify the inoculum, colony forming units (cfu) of FON were assessed by series dilution on PDA five days before use.

Bulb planting and inoculation

Narcissus bulbs from the low basal rot stock A (1% incidence) which had undergone HWT with Mirage, Orius, Storite, Bravo or without fungicide were planted in 20 cm diameter, 4 L capacity plastic plant-pots in a *Narcissus* growing medium consisting of a blend of sphagnum peat / horticultural sand (3:1, v/v) mixed with John Innes No.1 compost (1:1, v/v) and amended with ammonium nitrate (0.40 kg m^{-3}), potassium nitrate (0.75 kg m^{-3}), single super-phosphate (1.50 kg m^{-3}), ground chalk (2.25 kg m^{-3}), ground magnesian limestone (2.25 kg m^{-3}) and fritted trace elements WM 255 (0.40 kg m^{-3}). The growing medium was artificially inoculated with appropriate amounts of FON inoculum (1.25 and 12.5 g kg^{-1}) to give ‘low’ and ‘high’ levels of 1 x 10^4 and 1 x 10^5 colony forming units g^{-1} growing medium respectively. Five *Narcissus* bulbs were planted approx. 10 cm deep (measured to the base of the bulb) in each pot and there were eight replicate pots per fungicide treatment/inoculum level combination. HWT bulbs without fungicide were also treated with the biocontrol agent HDC F184 by dipping them in ‘slurry’ consisting of 5 g product, 1 L water and 25 g guar gum to aid adhesion. Inoculated control treatments consisting of HWT bulbs without fungicide were set up for each FON inoculum level. In addition, a set of uninoculated control treatments (no fungicide, no FON) associated with each FON inoculum level was also set up for a balanced experimental design. Planting was carried out over two days (01/10/13-02/10/13) and all pots were placed in saucers in a frost-free glasshouse on 03/10/13 with shading and watered from below twice weekly as appropriate. Pots were randomised in an 8-row and 14-column design over two parallel benches in the glasshouse such that a replicate pot of each treatment appeared in each column and row.

Plant and bulb assessments

Narcissus plants were assessed on four occasions (04/02/14, 19/02/14, 04/03/14 and 18/03/14) when height (length to longest leaf tip, mm), number of flowers, flower height (to bottom of spathe, mm) and flower diameter (when fully open, mm) were recorded. Another assessment on 01/04/14 recorded the proportion of plant leaves with chlorosis for each plant, which potentially indicated infection by FON. When most plants in the uninoculated control treatments had begun to senesce on 05/05/14, watering was halted and all the pots allowed todry-out for approx. 3 weeks before the bulbs were assessed for FON infection between 02/06/14 and 04/06/14. As before, the bulbs were bisected lengthways and basal rot incidence and severity recorded on the 10-point scale (Table 1).

Data analysis

After initial inspection of the data, plant and bulb assessments was analysed using Genstat to determine significant differences between treatments in terms of plant height and number of flowers per plant (square root transformed) on 18/03/14 (assessments at other times showed the same treatment effects), total number of leaves and proportion of leaves with chlorosis (angular transformed) on 01/04/14 and mean bulb disease score and proportion of bulbs (angular transformed) with low, medium and high severity on 02-04/06/14 (Table 1).

Results

Fungicide testing on agar

The three triazole fungicides HDCF F163, F164 and F165 reduced growth of all FON isolates on agar at all concentrations tested compared to the control cultures (no fungicide). Overall, there was a small significant variation in response to each fungicide between the eight isolates but, for clarity, growth reduction for each fungicide compared to the control (no fungicide) is presented over all eight FON isolates (Fig. 6). Reduction in FON growth varied between 41% for HDC F164 at 1 ppm to 100% for HDC F165 at 5-100 ppm. Maximum FON growth reduction for HDC F163 and HDC F164 was 82 and 80% respectively at 100 ppm. Therefore HDC F165 had greater activity than the other fungicides at all concentrations. MIC50 values were < 1 ppm for all three fungicides and HDC F165 had the lowest MIC95 value of 3.6 ppm which was comparable with the most effective fungicides tested in BOF 74 (Mirage, Agate and Orius, Table 2).

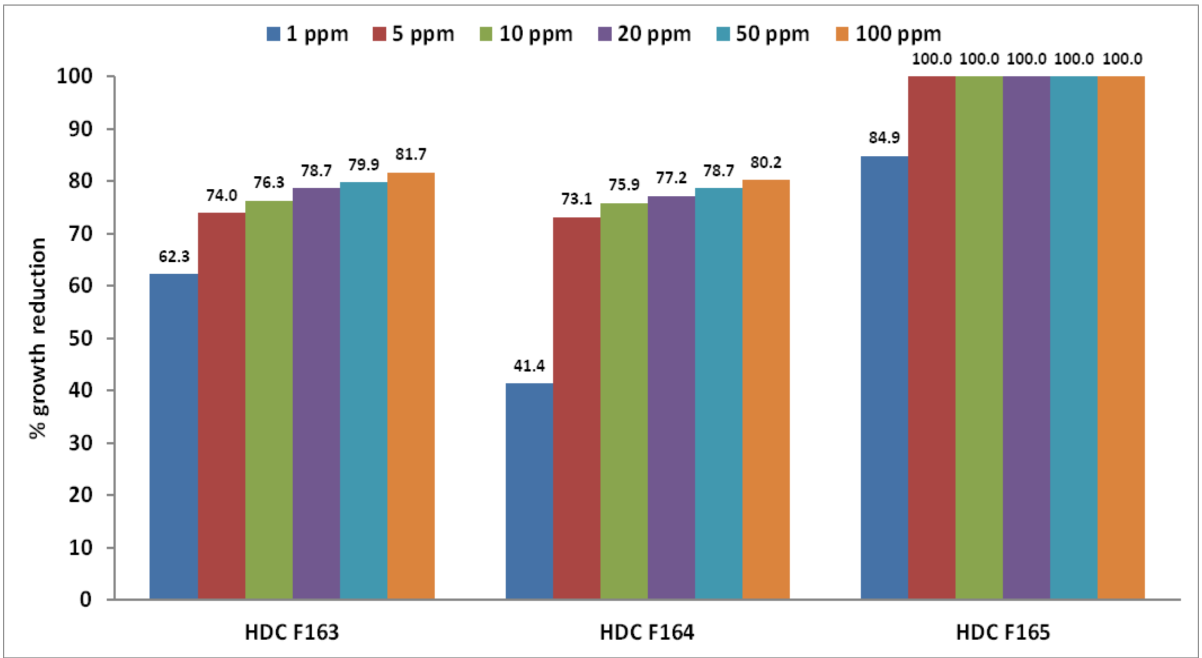

Figure 6. Percentage growth reduction of *F. oxysporum* f.sp. *narcissi* (FON) for six concentrations of three triazole fungicides on agar. Data are means (back-transformed data) across eight FON isolates (three replicates). Least significant difference (95% confidence level) =1.1% for angular transformed data.

Table 2. Minimum inhibitory concentration (MIC, calculated from regression analysis of growth reduction vs log fungicide rate) of fungicides tested in BOF 74 and BOF 74a required to reduce *F. oxysporum* f.sp. *narcissi* growth by 50% and 95%.

	Active ingredient (a.i.)	**50% MIC (ppm a.i.)**	**95% MIC (ppm a.i.)**
BOF 74 fungicides			
Agate	Tebuconazole + prochloraz	<1	<1
Mirage	Prochloraz	<1	<1
HDC F46	Coded product	<1	3
Storite (sensitive FON isolates)	Thiabendazole	2	5
Orius	Tebuconazole	<1	7
HDC F48	Coded product	<1	15
Cuprokylt	Copper oxychloride	35	119
Storite (tolerant FON isolates)	Thiabendazole	28	182
HDC F47	Coded product	<1	471
Signum	Pyraclostrobin + boscalid	12	>1000
HDC F49	Coded product	>1000	>1000
Comet	Pyraclostrobin	<1	>1000
HDC F54	Coded product	46	>1000
Bravo	Chlorothalonil	9	>1000
BOF 74a fungicides			
HDC F163	Coded product	<1	>1000
HDC F164	Coded product	<1	>1000
HDC F165	Coded product	<1	3.6

Fungicide testing on *Narcissus* bulbs/plants

Curative experiment

Following incubation of the *Narcissus* bulbs from the basal rot infected stock B (9% incidence) which had undergone HWT with fungicides, incidence of basal rot (>0 on the 10-point scale) in the bulbs was 19, 20, 57 and 60% for Bravo, Orius, Mirage and Storite respectively compared to 55% in the control (HWT with no fungicide). Incidence of basal rot in 40 spare bulbs which had not undergone either HW or fungicide treatment was 0%. Mean basal rot severity scores were 0.6, 1.2, 2.0 and 4.1 for Bravo, Orius, Mirage and Storite respectively compared to 4.8 for the control (HWT with no fungicide) and 0.0 for bulbs without HWT or fungicide. The percentage of bulbs with low basal rot severity (score 0-2, Table 1) was 87-95% for Bravo, Orius and Mirage but was much lower for Storite at 55% (Fig. 7). The percentage of bulbs with low basal rot severity was 60% for the HWT control bulbs (no fungicide, Fig. 7).

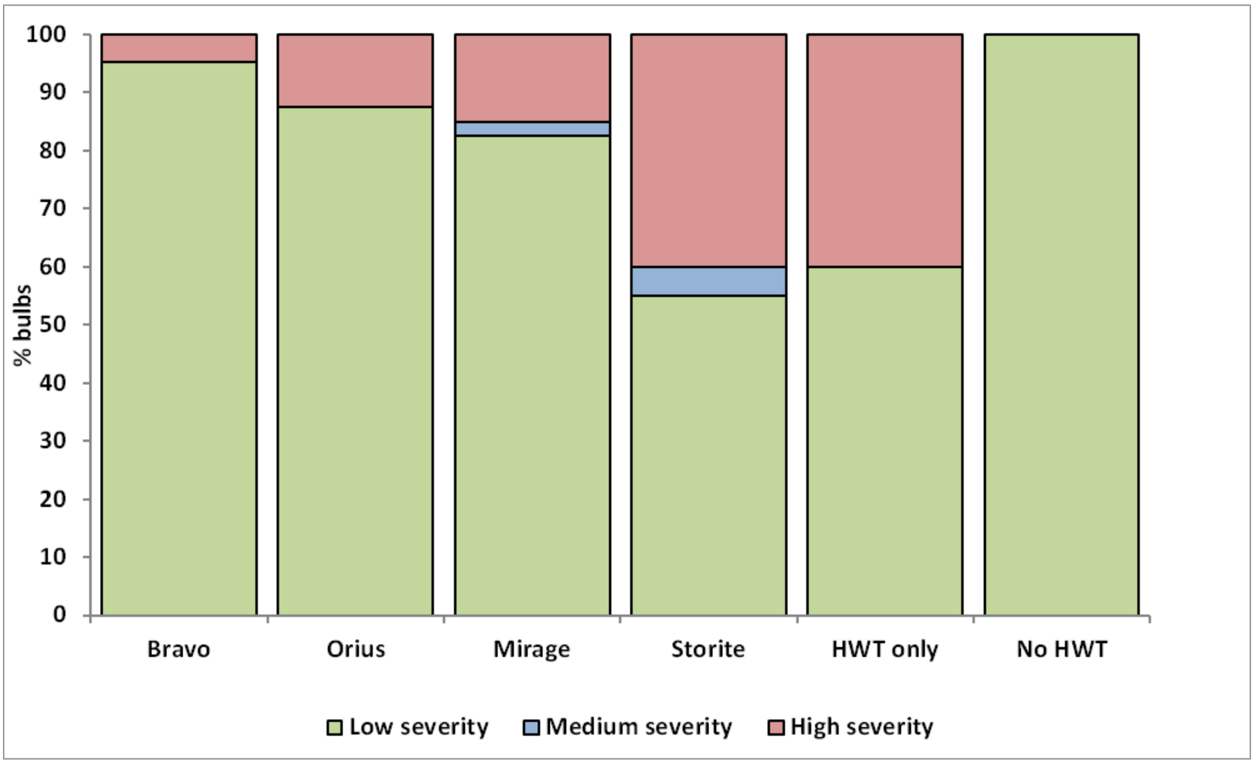

Figure 7. Percentage of Narcissus bulbs with low, medium and severe basal rot severity from a bulb stock with high initial disease incidence (9%) after HWT with different fungicides and incubation at 25°C for 60 days. HWT = hot water treatment only (no fungicide), No HWT = no hot water treatment (no fungicide).

Protectant experiment

Plant assessments

Plant height (18/03/14) was significantly reduced for control bulbs not treated with fungicide and planted in FON-inoculated compost compared with those planted in compost without FON (Fig. 8A, Table 3) but there was no difference between the two FON inoculum levels. This indicated that plant height was reduced by FON infection. Bravo and Mirage significantly increased plant height (407-448 mm) compared to the inoculated control treatments (349-363 mm) at both inoculum levels while Storite significantly increased height at the high FON inoculum level. Orius and the biocontrol treatment HDC F184 had no significant effect on plant height.

As expected for *Narcissus* bulbs grown in the greenhouse following HWT, few plants produced flowers with only 5-12% of plants flowering across all the fungicide treatments with the exception of Orius. For this treatment, 22 and 17% of plants produced flowers at the low and high FON inoculum levels respectively (Table 3). The mean number of flowers per plant was hence significantly increased for Orius at the low FON inoculum level compared to the corresponding inoculated control but not for the high level (Fig. 8B, Table 3). There was also an increase in flowers per plant for Bravo at the low inoculum level but again this was not significant. The biocontrol treated bulbs did not produce any flowers at all and there was no apparent effect of any treatment on flower diameter (data not shown). There was also no significant difference in flower production between inoculated and uninoculated controls suggesting that FON did not affect flowering for these first year bulbs.

The percentage of plants with leaf chlorosis (01/04/14) was significantly greater in the FON-inoculated control treatments (no fungicide) compared to the uninoculated control plants for both inoculum levels (Fig. 8C, Table 3) demonstrating that this symptom was a good indicator of FON infection (Fig. 9). All the fungicides significantly reduced leaf chlorosis compared to the appropriate inoculated control treatments for both inoculum levels while the biocontrol treatment was ineffective (Fig. 4C, Table 3).

Table 3. Effect of fungicides and the biocontrol agent HDC F184 on different plant growth parameters for HWT *Narcissus* bulbs planted in compost inoculated with *F. oxysporum* f.sp. narcissi (FON) at low (L) and high (H) inoculum levels. Means are for 40 plants per treatment (untransformed data). *indicates treatment is significantly different (5% level) from inoculated control of corresponding inoculum level following ANOVA analysis of transformed data.

Treatment	Plant height (mm)	% plants flowering[1]	No. of flowers / plant	% chlorotic leaves / plant
Storite L	378.97	5.0	0.051	7.4 *
Storite H	422.85 *	7.5	0.075	1.5 *
Bravo L	447.90 *	12.5	0.175	6.5 *
Bravo H	439.90 *	7.5	0.075	15.9 *
Mirage L	417.85 *	5.0	0.050	0.6 *
Mirage H	407.31 *	7.5	0.103	3.3 *
Orius L	384.20	22.5	0.250 *	3.7 *
Orius H	382.73	17.5	0.200	8.0 *
HDC F184 L	392.32	0.0	0.000	15.9
HDC F184 H	356.10	0.0	0.000	31.2
FON only L	363.38	5.0	0.050	16.2
FON only H	349.18	10.0	0.100	20.2
No FON (L)	472.73 *	7.5	0.100	1.0 *
No FON (H)	488.93 *	7.5	0.075	3.9 *

[1]no statistical analysis – directly related to no. of flowers / plant.

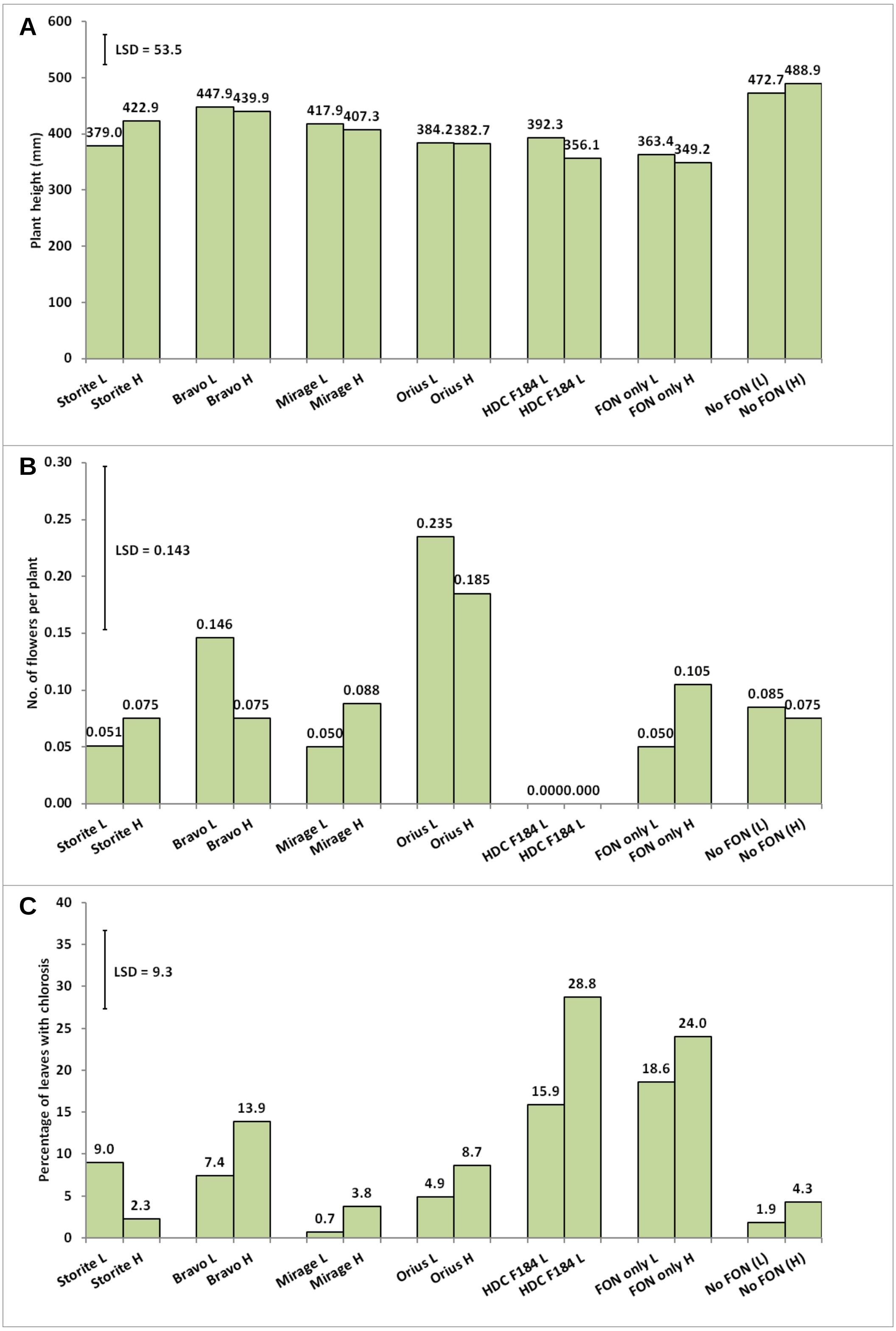

Figure 8. Effect of fungicides and the biocontrol agent HDC F184 on A) mean plant height (mm), B) mean number of flowers per plant (square root transformed) and C) mean proportion of leaves per plant with chlorosis (angular transformed) for HWT *Narcissus* bulbs planted in compost inoculated with *F. oxysporum* f.sp. *narcissi* (FON) at low (L) and high (H) inoculum levels. Data are means for 40 plants per treatment. Bar = Least Significant Difference (LSD, 5% level).

Figure 9. *Narcissus* plant growth on 01/04/13 showing stunted and chlorotic symptoms of infection by *F. oxysporum* f.sp. *narcissi* (FON) for the FON-inoculated control (left) compared to healthy foliage for Orius (centre) and the uninoculated control (no FON, right).

Bulb assessments

The incidence of basal rot in *Narcissus* bulbs planted in FON-inoculated growing medium ranged between 87 and 100% across all the treatments and inoculum levels (Table 4); disease pressure was therefore high. Disease incidence for bulbs planted in growing medium without FON (uninoculated control) was also high at 84% despite the level of incidence originally being recorded as 1%. Nevertheless, the severity of basal rot (mean disease score, proportion of bulbs in the low severity category) in the uninoculated control treatments (no FON) was significantly lower than in the inoculated control treatments at both inoculum levels (Table 4, Figs. 10, 11, 12, 13) and varied widely between the treatments allowing efficacy of fungicide performance to be determined. There was however, no significant difference in disease severity between high and low inoculum levels (mean score, proportion of bulbs in the low basal rot severity category).

Storite, Mirage and Orius significantly reduced the mean basal rot severity score for both FON inoculum levels ranging between 1.9 (Orius, low) and 3.9 (Storite, high) compared to 5.0 and 5.5 in the low and high inoculum untreated control treatments respectively (Table 3, Fig. 10). Of these three fungicides, Orius was the most effective resulting in the lowest disease severity score (1.9, low; 3.0, high) at both inoculum levels. Bravo did not significantly reduce basal rot severity and the biocontrol HDC F184 actually significantly increased severity at both FON levels (Table 4, Fig. 10) compared to the inoculated controls.

Storite, Mirage and Orius treatments also resulted in a significantly larger proportion of bulbs in the low basal rot severity category (0-2) ranging from 45 (Storite, low/high) to

77.5% (Orius, low) compared to 22.5% in both the low and high inoculum untreated control treatments respectively (Table 4, Figs. 11, 12). Of the four fungicides, Orius was again the most effective resulting in the highest proportion of bulbs in the low severity category (low, 77.5; high, 60.0; Fig. 12) which was significant for both inoculum levels. Bravo also increased the proportion of bulbs in the low disease severity category compared to the inoculated control treatments (low, 30; high, 27.5) but this was not significant while the biocontrol HDC F184 resulted in a reduced proportion of bulbs in the low severity category.

Table 4. Effect of fungicides and the biocontrol agent HDC F184 on basal rot incidence (% FON) and severity for HWT *Narcissus* bulbs planted in compost inoculated with *F. oxysporum* f.sp. *narcissi* (FON) at low (L) and high (H) inoculum levels. Data are means for 40 plants per treatment (untransformed data). Shaded cells indicate treatment is significantly different (5% level) from inoculated control of corresponding inoculum level following ANOVA analysis of severity score data (no transformation) and total proportion of bulbs in low, medium and high basal rot severity score categories (after angular transformation).

	% *Narcissus* bulbs in different disease severity score categories															
	LOW				**MEDIUM**				**HIGH**						**% FON[1]**	**Mean Severity Score**
	0	**1**	**2**	**Tot**	**3**	**4**	**5**	**Tot**	**6**	**7**	**8**	**9**	**10**	**Tot**		
Storite L	7.5	27.5	10.0	**45.0**	10.0	5.0	5.0	**20.0**	7.5	7.5	17.5	0.0	2.5	**35.0**	92.5	3.5 *
Storite H	7.5	30.0	7.5	**45.0**	7.5	5.0	5.0	**17.5**	10.0	7.5	12.5	7.5	0.0	**37.5**	92.5	3.9 *
Bravo L	10.0	15.0	5.0	**30.0**	2.5	0.0	12.5	**15.0**	15.0	22.5	10.0	2.5	5.0	**55.0**	90.0	5.0
Bravo H	7.5	2.5	17.5	**27.5**	2.5	7.5	12.5	**22.5**	7.5	10.0	22.5	10.0	0.0	**50.0**	92.5	5.2
Mirage L	5.3	34.2	7.9	**47.4**	2.6	0.0	15.8	**18.4**	7.9	7.9	7.9	7.9	2.6	**34.2**	94.7	3.7 *
Mirage H	5.1	33.3	12.8	**51.2**	10.3	5.1	2.6	**18.0**	5.1	7.7	10.3	5.1	2.6	**30.8**	94.9	3.6 *
Orius L	12.5	52.5	12.5	**77.5**	2.5	2.5	10.0	**15.0**	5.0	0.0	0.0	2.5	0.0	**7.5**	87.5	1.9 *
Orius H	12.5	35.0	12.5	**60.0**	2.5	2.5	7.5	**12.5**	12.5	10.0	5.0	0.0	0.0	**27.5**	87.5	3.0 *
HDC F184 L	2.6	0.0	5.3	**7.9**	2.6	2.6	5.3	**10.5**	21.1	2.6	18.4	23.7	15.8	**81.6**	97.4	7.2
HDC F184 H	0.0	0.0	14.6	**14.6**	2.4	2.4	12.2	**17.0**	17.1	12.2	19.5	17.1	2.4	**68.3**	100.0	6.3
FON only L	0.0	10.0	12.5	**22.5**	5.0	12.5	10.0	**27.5**	20.0	17.5	7.5	5.0	0.0	**50.0**	100.0	5.0
FON only H	0.0	7.5	15.0	**22.5**	2.5	7.5	17.5	**27.5**	10.0	17.5	12.5	0.0	10.0	**50.0**	100.0	5.5
No FON (L)	15.0	57.5	7.5	**80.0**	0.0	0.0	0.0	**0.0**	5.0	2.5	10.0	2.5	0.0	**20.0**	85.0	2.2 *
No FON (H)	15.8	73.7	0.0	**89.5**	2.6	0.0	0.0	**2.6**	2.6	0.0	5.3	0.0	0.0	**7.9**	84.2	1.4 *

[1]no statistical analysis

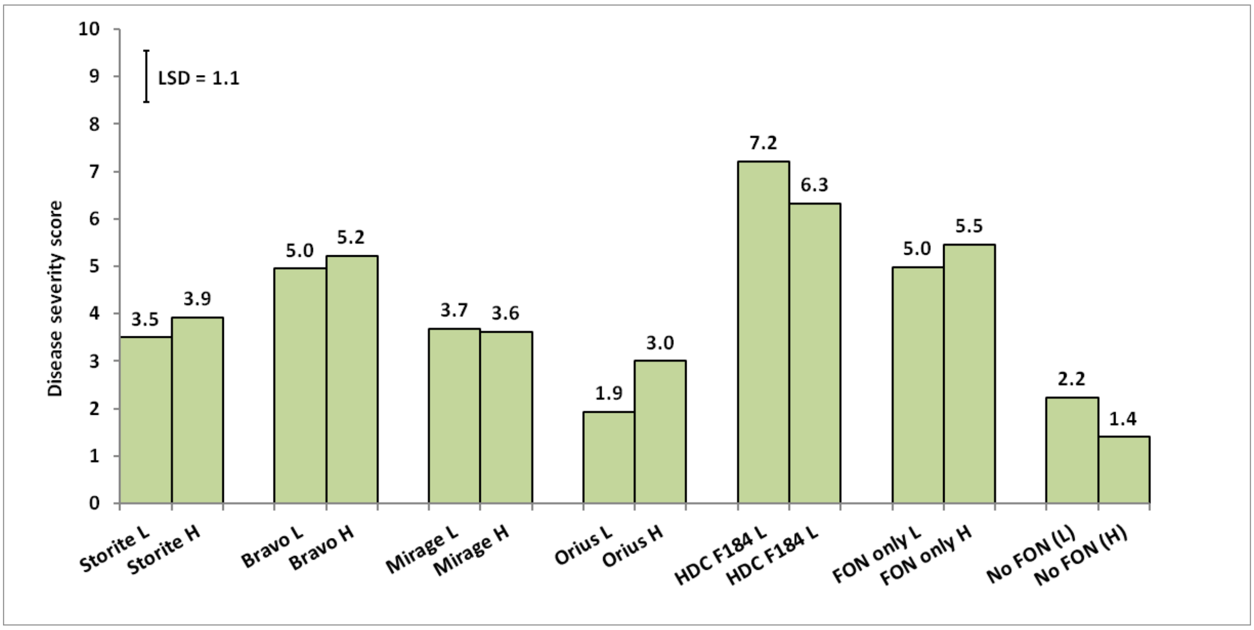

Figure 10. Effect of fungicides and the biocontrol agent HDC F184 on basal rot severity score for HWT *Narcissus* bulbs after planting in compost inoculated with *F. oxysporum* f.sp. *narcissi* (FON) at low (L) and high (H) inoculum levels. Data are means for 40 plants per treatment. Bar = Least Significant Difference (LSD, 5% level).

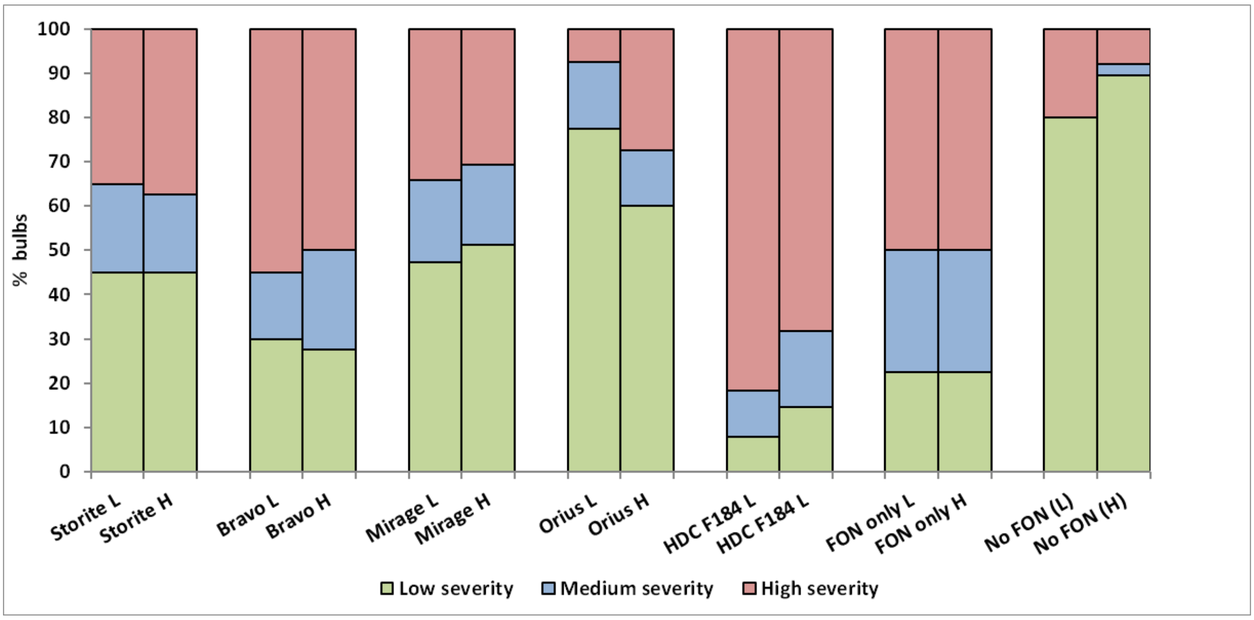

Figure 11. Effect of fungicides and the biocontrol agent HDC F184 on percentage of HWT *Narcissus* bulbs in different disease severity categories after planting in compost inoculated with *F. oxysporum* f.sp. *narcissi* (FON) at low (L) and high (H) inoculum levels. Data are means for 40 plants per treatment.

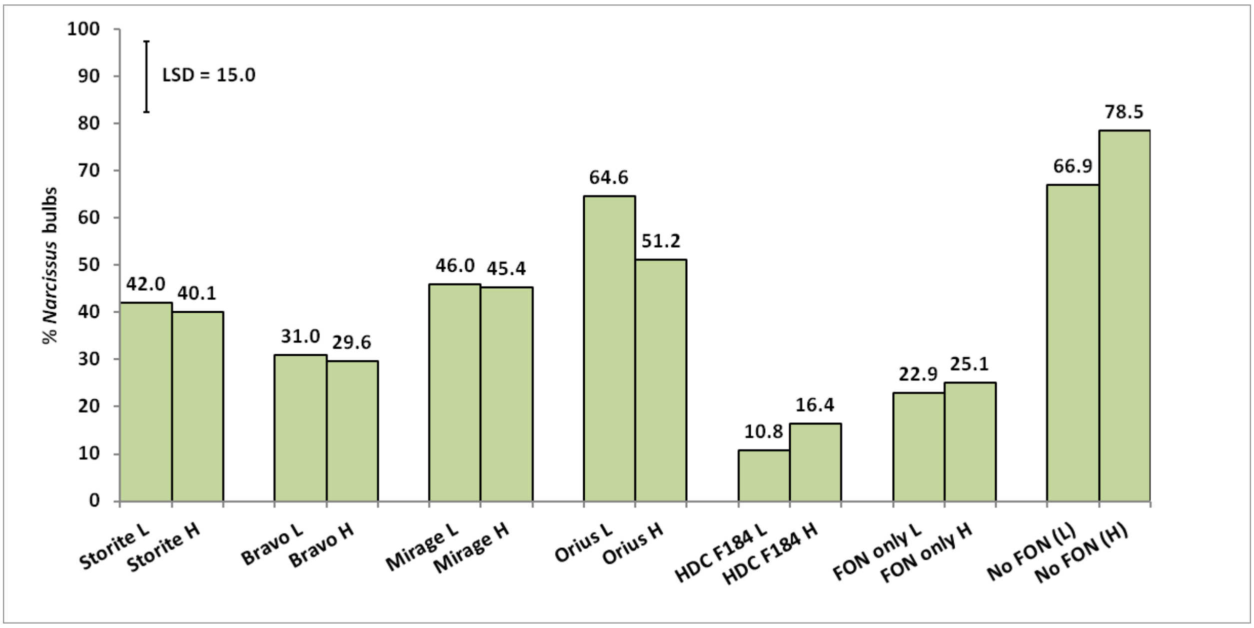

Figure 12. Effect of fungicides and the biocontrol agent HDC F184 on percentage of HWT *Narcissus* bulbs with low basal rot severity (0-2 disease score) after planting in compost inoculated with *F. oxysporum* f.sp. narcissi (FON) at low (L) and high (H) inoculum levels. Data are means for 40 plants per treatment (angular transformed). Bar = Least Significant Difference (LSD, 5% level).

Figure 13. *Narcissus* bulbs showing symptoms of infection by *F. oxysporum* f.sp. *narcissi* (FON) for the FON-inoculated control (left) compared with Orius (centre) and the uninoculated control (no FON, right)

Discussion

Fungicide testing on agar

The three triazole fungicides tested inhibited growth of FON at all concentrations, but it was clear that HDC F165 was the most effective. MIC50 and MIC95 values of <1 and 3.6 ppm a.i. respectively for this product were comparable with those for Agate (tebuconazole + prochloraz), Mirage (prochloraz), HDC F46, Storite (thiabendazole) and Orius (tebuconazole) in BOF 74 (Clarkson, 2012). HDC F165 therefore represents a potential future alternative to the currently approved fungicides (Storite, Bravo) although would first need evaluating on bulbs with HWT.

Fungicide testing on *Narcissus* bulbs/plants

Curative experiment

Although thiabendazole is described as a systemic, curative and protectant fungicide, it has generally been assumed that Storite works in a protective, rather than curative, way when applied to *Narcissus* bulbs, though there does not appear to be data that exclude a curative role. This preliminary experiment aimed to test the potential curative effect of effect of fungicides applied in HWT for a *Narcissus* bulb stock with a high initial basal rot incidence of 9%. In contrast to Storite, the three fungicides Bravo, Orius and Mirage reduced basal rot development compared to the control (HWT with no fungicide), indicating that these products have some value in treating bulb stocks known to be contaminated with FON. Although the experimental approach here may have demonstrated some curative activity, it is doubtful whether a fungicide could prevent disease development in bulbs with an established internal FON infection (in the basal plate and/or bulb scales) at the time of treatment; indeed in HDC project BOF 43a (Hanks, 2003) it was shown that little of the a.i. reaches the centre of the bulb. In the current project, the lack of curative action was evidenced by the general increase in FON incidence from the original value of 9%. Hence, the observed ‘curative’ activity is more likely to be associated with a preventative mode of action whereby spores carried on or just within the basal plate are killed and any bulb to bulb disease spread is inhibited. Nevertheless these preliminary results suggest that fungicide treatment of bulb stocks with high initial incidence of FON may be worthwhile, and therefore requires further investigation in a fully replicated experiment.

A confounding factor in experiments involving bulb dip treatments (including HWT) is that control dips (i.e. where bulbs are dipped in plain water without a fungicide) are likely to spread / increase levels of the target disease. Hence bulb dip treatments that do not incorporate a proven fungicide against FON are always discouraged, whether they are HW or ‘cold dip’ treatments. HWT is always advised before planting stock bulbs because the high temperatures control stem nematode, though again a proven fungicide against FON will need to be added, so omitting HWT entirely is not a realistic ‘control’ in these experiments. In the present case the HWT process, in the absence of a fungicide, indeed promoted FON infection (with a final incidence of 55% compared with 0% for bulbs that received no HWT).

The bulbs which received no HWT or fungicide had a final basal rot incidence of 0% which was unexpected as initial assessment of incidence was 9%. This is difficult to explain but may have been due to the relatively small number of bulbs used in the experiment.

Protective experiment

This experiment tested the protectant effect of fungicides applied in HWT for *Narcissus* bulbs planted in growing medium inoculated with two levels of FON. Growth assessments of plants derived from untreated bulbs (inoculated controls) indicated that FON infection was associated with a reduction in plant height and more importantly chlorosis/yellowing of the leaves. Quantifying these parameters showed that Bravo and Mirage significantly increased plant height compared to the inoculated controls whilst all four fungicides, Bravo, Mirage, Orius and Storite significantly reduced the proportion of chlorotic leaves, indirectly indicating a decrease in FON severity. The biocontrol treatment HDC F184 however had no effect on plant growth symptoms associated with basal rot. Interestingly, although there was no difference in the number of flowers produced between inoculated and uninoculated control plants with no fungicide treatment, Orius significantly increased the number of flowers per plant at the low inoculum level. There is no obvious explanation for this phenomenon which would have to be investigated further to be confirmed.

Following the plant growth assessments, direct assessment of basal rot was carried out on the bulbs once the foliage had died down naturally. Incidence of basal rot was high (85-100%) across all treatments for bulbs planted in FON-inoculated growing medium but also for the uninoculated controls (84%). This suggested that although basal rot incidence was originally recorded at only 1% for the bulb lot used, some natural FON inoculum was present which resulted in infection of the uninoculated bulbs over the course of the experiment. This may have been promoted by the HWT itself as suggested by the results from the curative experiment, but the lack of a non-HWT uninoculated bulb treatment in this main experiment meant that this could not be confirmed. Cross contamination of the uninoculated controls from the inoculated treatments was also unlikely as all pots were contained in saucers and watered from below.

Despite a high disease incidence overall, the severity of basal rot in *Narcissus* bulbs was much lower in the uninoculated control treatments compared to the inoculated controls for both inoculum levels, and there were also clear differences between fungicide treatments. Storite, Mirage and Orius all significantly reduced basal rot severity compared to the inoculated controls. Of these, Orius was most the effective with the lowest disease severity score and the greatest percentage of bulbs with a low severity score. Despite some indication of an effect based on the plant growth assessments, Bravo did not significantly reduce basal rot severity (although bulbs had less basal rot than the inoculated controls), while the biocontrol agent was ineffective.

Overall therefore, Orius (containing tebuconazole) and Mirage (containing prochloraz) represent very effective alternative fungicides to the currently approved Storite and Bravo for control of basal rot.

Fungicides potentially available for the control of *Narcissus* basal rot

Tebuconazole

Tebuconazole has previously been shown to have activity against other *F. oxysporum formae speciales* pathogenic to other crop plants such as onion (Ozer & Koycu, 1998), strawberry (Lin et al., 2009) and shallot (Sintayhe et al., 2011). Some tebuconazole products have EAMUs as foliar sprays for smoulder (*Botrytis narcissicola*) on daffodils grown for galanthamine production as well as for treating onion sets, although this latter use is officially for protection against white rot (*Sclerotium cepivorum*) rather than *F. oxysporum*. Based on the results of this project and previous work (Clarkson, 2012), the HDC are currently pursuing an EAMU for a tebuconazole product for use in HWT of *Narcissus* bulbs (HDC, personal communication, 2014). A justification for this use was prepared in June 2014 (Hanks, 2014) and the outcome is awaited. With grower backing, the case emphasised the need for any 'bulb dip' approval to cover the full range of treatment conditions used, as was the case in the earlier SOLAs for thiabendazole and chlorothalonil products. These conditions should range from a short (15 minute) cold dip, which can be a useful adjunct to HWT, through 'standard' HWT (currently 3¼ hours at 44.4°C), to the increasingly rigorous HWT regimes now being adopted by UK growers (often up to 4 hours at 48°C). Tebuconazole has recently been reviewed in the EU with current approval until 2019.

Prochloraz

Prochloraz was previously identified as having activity against FON and other pathogens implicated in daffodil neck rot (Hanks & Linfield, 1996) and preliminary field trials where it was applied in HWT suggested that it could enhance crop vigour and bulb yields without adverse effects on the crop. Previously, prochloraz (as Sportak 45) was available in the UK through a SOLA for use in bulb dips (including HWT) at a rate of 1 L in 1000 L water, apparently based on Dutch usage. The results in this project therefore confirm the activity of prochloraz against FON. Dutch trials have shown that a low-rate tank-mix of prochloraz, thiabendazole and captan in HWT (for 2 h at 43.5°C) gave good results for all cultivars in yield, disease control and skin quality (Vreeburg et al., 1993; Boskamp, 1993), a recommendation still in use today in Holland (P. Venderbosch, personal communication, 2012). Prochloraz was recently reviewed under new EU regulations in 2012 and as a result all products are currently going through re-registration. It is understood that manufacturers are applying for a label use for prochloraz based fungicides for use in bulb dipping in the Netherlands (HDC, personal communication, 2014). This may present an opportunity for a mutual recognition EAMU for UK growers. However, the use in the UK would have to be identical to that in the Netherlands which may only be a 15 minute cold water dip. This may be appropriate for some uses on other flower-bulbs in Holland but UK growers have confirmed that this would be inappropriate for HWT use in UK daffodil production. The HDC are in discussion with agrochemical companies to identify the best way forward for UK narcissus growers.

Tebuconazole + prochloraz

Tebuconazole and prochloraz are available in combined formulations such as Agate. The HDC has indicated a willingness to investigate an EAMU for Agate in addition to the individual products, though this too will be dependent on the outcome of the current review of prochloraz (HDC, personal communication, 2014).

Thiabendazole

Thiabendazole fungicides have for many years been considered the most consistent products to control daffodil basal rot in trials (Hanks, 2013). Storite Clear Liquid which is currently approved at a full rate of 1.25 L of product per 1000 L of water (along with Tezate 220 SL) for HWT treatment of *Narcissus* bulbs continued to show good activity against FON in this study. However, results from the previous project BOF 74 (Clarkson, 2012) demonstrated that some isolates exhibit resistance to this fungicide, though their prevalence is unknown. This demonstrates the problems of relying on one or two actives for FON control and why alternatives are urgently required. Moreover, approvals for these two thiabendazole products will expire at the end of 2015 and there are currently no plans for the manufacturers to apply for re-registration, except for possibly a co-formulation of thiabendazole and imazalil (HDC, personal communication, 2014). However, imazalil was shown to have some phytotoxic effects on narcissus and inconsistent control of basal rot in HDC project BOF 31a (Hanks & Linfield, 1996).

Chlorothalonil

The chlorothalonil fungicide Bravo 500 was identified as an alternative to thiabendazole fungicides in HDC projects BOF 61a and 61b (Lole et al., 2010; Hanks, 2012), and, as a result, SOLAs were granted for the use of some chlorothalonil fungicides in daffodil HWT. Bravo 500 has been widely used for the purpose at a full rate of 1.0 L of product per 1,000 L of water. However, in the present project it reduced basal rot severity only slightly (and non-significantly) in the pot tests, and, compared to the other fungicides tested, its performance was relatively weak. However, Bravo 500 did show some potential activity in the curative experiment, although this would need to be confirmed in a larger, replicated trial. Bravo 500 was also found to be less effective than the other fungicides in agar tests carried out previously (Clarkson, 2012). Nevertheless, chlorothalonil fungicides provide an alternative fungicide for HWT use but should be used at the full rate based on the results here. Of the three products with SOLAs, Bravo 500, LS Chlorothalonil and Life Scientific Chlorothalonil, approvals currently expire on 30/04/20, 31/05/15 and 30/04/20 respectively.

Copper oxychloride

Although not tested in the present project, the copper oxychloride fungicide Cuprokylt FL was shown in project BOF 74 to have potentially useful activity against FON, though at higher concentrations than required by tebuconazole or prochloraz. The authors also requested the HDC to investigate an EAMU for copper oxychloride fungicides. This enquiry is currently on hold as these products are going through a change of ownership (HDC, personal communication, 2014).

Prothioconazole

Although not tested in projects BOF 74 or 74a, the HDC have drawn attention to a Dutch approval for this a.i. as Rudis. This refers only to a 15 minute dip for bulbs as a control for *F. oxysporum* (HDC, personal communication, 2014). Discussions with growers suggested that a cold dip might still be a useful alternative treatment but unfortunately the manufacturer has confirmed that they will not be supporting a bulb dip use in the UK.

Biocontrol materials

The biocontrol treatment HDC F184 had no effect on basal rot in this study despite some previous evidence of activity (Noble, 2012). The product was applied as a guar gum based dip as a means of ensuring adhesion to the bulbs which may have affected efficacy. However, preliminary work had showed that guar gum has no effect on spore viability. Much earlier, research in the Netherlands and the UK had shown that *Minimedusa polyspora*, *Streptomyces* species and other micro-organisms were effective in controlling FON in small-scale experiments (Beale & Pitt, 1990; Hiltunen et al., 1995), although attempts to adapt the technique to field-scale use (albeit using rather simplistic application methods) in HDC project BOF 6 (Pitt, 1991) were unsuccessful. Further biocontrol products are now being approved and coming onto the market so this approach to control should continue to be pursued in future work in the interest of maintaining a diverse range of control approaches to basal rot.

Towards a future strategy for basal rot management

This strategy assumes that thiabendazole and chlorothalonil-based products will remain available to UK narcissus growers for bulb dips in the short to mid-term and that tebuconazole will gain approval in the near future. This still only allows a very limited number of options to growers, and hence as stated previously, new chemical and biological control options should continually be assessed. It must also be considered that in the medium-long term, further EU legislation may restrict or remove the availability of these fungicide products.

- As a minimum fungicide strategy, tebuconazole (when approved), thiabendazole or chlorothalonil should be alternated at full-rate in HWT every time a stock of bulbs is lifted and re-planted. Thiabendazole fungicides may not be applied more than once in any one year and is not currently permitted for use in the Isles of Scilly.
- In basal rot-susceptible *Narcissus* cultivars, stocks where basal rot is a particular problem, or where bulb stocks are to be left in the ground for more than two years, a post-lifting fungicide application could be applied. This may be as a 15-minute cold dip or an on-line spray application; in current UK SOLAs, ‘bulb dip’ applications are construed as including short, cold dips as well as HWT, while on-line spray applications require specific approval. Thiabendazole and chlorothalonil can be used as post-lifting dips (bearing in mind the above restrictions on thiabendazole use), while tebuconazole is untested for this application. Only thiabendazole may currently be used as an on-line bulb spray. In the absence of an effective nematicidal biocide

to add to cold dips, cold dips should not be used where a stock is known (or suspected) to be infested with stem nematode (eelworm) because a bulb soak will spread it.

- Attention to hygiene is also important, particularly as the chlamydospores of *F. oxysporum* (and the 'wool' phase of stem nematode) can survive for many years in a dehydrated state. Each year before use, the bulb-handling equipment, bulb stores and HWT facilities should be cleaned physically and then with a suitable disinfectant. The importance of removing biological contamination from HWT systems at the start of the treatment season, using FAM 30 (or other suitable iodophore biocide) was demonstrated in HDC project BOF 70 (Hanks, 2010).
- With such limited fungicide options available, basal rot-resistant cultivars should be used wherever possible, and full use should be made of non-pesticide options for managing basal rot, such as prompt bulb drying after lifting, use of optimal bulb storage conditions, delaying re-planting when soil temperatures are still high, and avoiding over-fertilising with nitrogen.

If other fungicides become available for this use, then growers should aim to largely substitute them for thiabendazole because of the known resistance to it of some FON isolates.

With such limited options available, other research should be considered.

Research recommendations for improving *Narcissus* basal rot management

- Continue to test other appropriate fungicides / disinfectants and biocontrols for narcissus basal rot control as they become available. Where effective products are found, refine the protocols for using them under real-life conditions (e.g. check stability under HWT conditions and examine their effectiveness as short, cold dips).
- Agrochemical companies are not attracted to pesticide applications via HWT because of concerns over high temperatures, dip volumes, spent dip disposal and operator safety. Alternative delivery systems should be investigated, that could deliver in a five-year period.
- Irrespective of the methods adopted for managing basal rot, we need a better understanding of the basal rot (FON) pathogen and its isolates (fungicide resistance, aggressiveness and the significance of other *formae specialis*), and of the basis of resistance/tolerance to basal rot in narcissus and its cultivars and why this resistance breaks down over time.

Conclusions

- The fungicide HDC F165 was the most effective of three triazoles tested in reducing the growth of FON on agar and represents a potential alternative to the currently approved fungicides (Storite, Bravo) pending evaluation on bulbs in HWT.
- In a preliminary experiment, Bravo (chlorothalonil), Orius (tebuconazole) and Mirage (prochloraz) reduced basal rot development in a *Narcissus* bulb stock with a high initial basal rot incidence (9%) but Storite (thiabendazole) had no effect. Results also suggested that HWT of bulbs promoted FON infection development.
- Orius (tebuconazole), Mirage (prochloraz) and Storite (thiabendazole) gave good control of basal rot for HWT bulbs planted in growing medium inoculated with FON with Orius (tebuconazole) being the most effective.
- HDC are currently pursuing an EAMU for tebuconazole for use in HWT of *Narcissus* bulbs.
- Approval for thiabendazole will be lost at the end of 2015 and there are currently no plans from manufacturers to apply for re-registration of this product except for possibly a co-formulation of thiabendazole and imazalil.
- The biocontrol agent HDC F184 showed no activity against basal rot. However, new biological products which are coming onto the market should be evaluated in order to develop a range of options for basal rot control.

Knowledge and Technology Transfer

An article will be prepared for HDC News in late summer or autumn 2014. This will include the revised management strategy and research recommendations.

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